

# Dextran-magnetic particles as a new MR contrast agent. For selective MR imaging of brain metastases

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## Summary and Conclusions

MR (magnetic resonance) imaging has proven to be a very sensitive diagnostic tool for the detection of cerebral lesions such as tumor metastases. These malignant lesions are currently depicted on account of their blood-brain barrier disruption and increased water content. However, the images lack specificity and it is often difficult to make a clear discrimination between neoplastic tissue and associated edema, i.e. water accumulation in the surrounding tissue due to leakage through the disrupted bbb. The diagnostic sensitivity of MR imaging in the detection of tumors may be improved by specific contrast enhancement. This can be effectuated by selective targeting of a MR contrast agent using specific immunological carriers, which is the subject of this thesis.

In **Chapter 1**, an introduction on this thesis is given. The dependency of signal intensity in MR imaging on the $T_1$ and $T_2$ relaxation times is explained, and the currently available MR contrast agents and their capabilities to alter tissue contrast are described. It is also discussed how monoclonal antibodies and liposomes may be used as selective delivery vehicles. Furthermore, primary brain tumors and tumors metastatic to the brain are discussed in view of the disrupted blood-brain barrier (bbb) associated with these malignant tissues.

In **Chapter 2**, various iron-oxide containing compounds were assessed as a potential MR contrast agent. Dextran-magnetite particles (DMP) clearly proved to have superior relaxation properties, predominantly affecting $T_2$, and were therefore selected for further study. The *in vivo* biodistribution after i.v. injection was assessed for the brain, kidney and liver in rats. It was found that DMP was rapidly and predominantly taken up by liver Kupffer cells. It appeared however, also that the particles were able to localize in those areas of the brain where the bbb was experimentally disrupted. To this end, a freezing injury to the rat cerebral cortex was used as a model of bbb-disruption. The presence of DMP in the blood circulation and the liver could be easily detected in MR imaging as a result of the strong decrease of signal intensity.

In **Chapter 3**, DMP was further investigated for its potential in the selective contrast-enhancement of lesions with a local bbb-disruption as induced by the freezing injury to the cerebral cortex. The biodistribution of i.v. injected DMP was studied using atomic absorption spectrophotometry, electron microscopy and MR imaging. One hour after injection, focal accumulation of the particles in capillary endothelial cells in the freezing lesion could be demonstrated by electron microscopy. MR imaging revealed the presence of the freezing lesion as a well defined dark spot, resulting from the extravasation of DMP. *In vivo*, the relaxivity of DMP, located in endothelial cells, was found to be significantly reduced as compared to that determined *in vitro* for water phantoms, probably as a result from a more restricted diffusion of water protons. Nevertheless, it was concluded that DMP can be used as a suitable contrast agent for the selective visualization of cerebral lesions.

In **Chapter 4**, the use of monoclonal antibodies as the vehicle for selective

delivery of DMP is described. Human peripheral blood lymphocytes (PBL's) were chosen as a model to investigate possible monoclonal antibody (moab)-mediated contrast enhancement of conjugated DMP. Using anti-L as a specific moab, the *in vitro* labeling resulted in a strong and specific signal reduction of the PBL suspensions, caused predominantly by a great specific increase of $R_2$ with a small but significant specific increase of $R_1$. The $R_1$ was found to decrease with increasing field strength. On the contrary, control experiments using MOC-31 as a non-specific (control) moab did not result in a increase of $R_2$ and signal reduction. DMP-moab conjugates therefore have great potential for the selective contrast enhancement of target cells in MR imaging.

In **Chapter 5**, the use of an alternative vehicle for selective DMP delivery is discussed. Human peripheral blood mononuclear cells (PBMNC's) were incubated with DMP encapsulated liposomes or magnetoliposomes. It was found that DMP could be efficiently incorporated into these cells. Electron microscopy revealed the presence of DMP in cells mainly in phagosomes and secondary lysosomes. DMP-labeled PBMNC's showed a strong increase of the transverse relaxation rate (up to 16.6 $s^{-1}$ for 5 x $10^7$ cells/ml) and, accordingly, a great loss of signal intensity in MR imaging. The fraction of DMP containing PBMNC's could be enriched by magnetic cell separation. The major population of the DMP containing cells proved to be monocytes. When peripheral blood lymphocytes depleted of monocytes were used for labeling, DMP uptake could also be observed in these cells. Conceivably, the *ex vivo* labeling of PBMNC's with magnetoliposomes has a great potential for the selective MR imaging of *in vivo* cell trafficking, e.g. in the monitoring of lymphocyte infiltration in tumors.

In **Chapter 6**, an animal model is developed which may be used for the further *in vivo* evaluation of the selective targeting of DMP to tumors, either by DMP-conjugated specific moab's or by DMP-labeled tumor-infiltrating PBMNC's. The human small cell lung carcinoma (SCLC) cell line GLC-28 was selected for xenotransplantation into nude rats. A subcutaneous (s.c) and two intracerebral (i.c., in the cerebral cortex and lateral ventricle of the brain) models of xenografted GLC-28 tumor were established. In all the three models *in vivo* localization of moab MOC-31 occurred within 4 hours after i.p. injection, with maximal binding at 24 h after injection. In addition it was noticed that a pronounced tumor infiltration of predominantly NK cells was observed for s.c. and intraventricular xenografts, but not for the GLC-28 tumors xenografted in the cerebral cortex. The nude rat/GLC-28 xenograft model appears to be a suitable model to investigate selective in vivo targeting of DMP.

Finally, in **Chapter 7**, several aspects of the presented studies where a more in-depth consideration was justified are elucidated. Some drawbacks which emerged from the studies are indicated, and suggestions for further research are given.

From these investigations, it is concluded that MR imaging in combination with the selective targeting of DMP has clinical potential for the non-invasive, improved diagnosis of (brain) metastases.